1-1-2005

Community structure and distributions of two terrestrial salamanders : a pluralistic approach

Saad Arif
Iowa State University

Community structure and distributions of two terrestrial salamanders: A pluralistic approach

by

Saad Arif

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

Program of Study Committee:
Dean C. Adams, Major Professor
Nicole Valenzuela-Castro
Dan Ashlock

Iowa State University

Ames, Iowa

2005

Graduate College
Iowa State University

This is to certify that the master's thesis of

Saad Arif

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 2:

ACKNOWLEDGEMENTS

First and foremost I would like to thank my advisor Dean Adams, who in my opinion at least, played his role par excellence. He provided ideas and encouragement when I needed it most. It has been a truly a fulfilling experience working with him for the past two years. Working with Dean has aided in developing my skills as a better biologist not only by asking creative questions but also thinking about those questions more quantitatively. I also appreciate the role of my committee members Nicole Valenzuela and Dan Ashlock for constructive input at the onset of the project and their comments and advice on the final version of this work. I would also like to thank my undergraduate advisor Thomas Rosburg on whose suggestion and friendly persuasions I decided to undertake a masters at Iowa State University. Dr. Jim Christansen is also deserving of thanks for being responsible as person who turned my interests from plants to herps.

There are several other people who helped me immensely directly and indirectly towards the culmination of my degree and this thesis. Those directly involved with the project include Addison Wynn from the Smithsonian Institution and Steve Rogers from the Carnegie Museum of Natural History. Both aided in providing specimens in an extremely timely fashion and I am much indebted to both. Several people stepped up to the plate in areas where I had minimal experience and their help has been indispensable. Pipat Rengusang and Robert Hijmans in particular provided help and insight with GIS software. A lot of the work done with GIS proved to be the last piece of the puzzle that helped me discern the bigger picture. Greg Courtney and Greg Curler were more than helpful when I first began identifying stomach contents. Their expertise and eagerness to help proved invaluable.

People that were involved indirectly include many friends and colleagues whose presence and encouragement helped me keep my feet firmly planted on the ground. Mike Collyer, Jen Deitloff, Erin Myers and Chris Holden were all equally reliable in dispensing ideas and providing criticisms as they were in being there when I just needed to 'goof-off'. Nabeel Hamid and Shayan Hussain, though not biologists were always willing to question my ideas, offering new perspectives and doing a bit of proof-reading every now and then. Their friendship, as before this, has been much appreciated throughout my time as a master's

student and I hope will continue to be so after this. Janani Krishnan and Shivani Todi have both been instrumental with financial support through the early years of my academic career. I would also like to thank both Italo Calvino and Franz Kafka. The works of both deceased writers has been a constant source of inspiration when there was little or none.

Finally, I wish to thank my parents and both my siblings. My brother and mother, Fawad Arif and Shamim Arif whose efforts in particular have been tantamount to heroic in getting me to where I am today. I thank all my family who have believed in me thus far and encouraged me at every tiny step even when to explain the significance of my work. That love and belief in a person is unconditional and indefatigable.

ABSTRACT

A goal of evolutionary ecology is to understand the forces that generate ecological communities and maintain species boundaries. Although the effects of particular biotic and abiotic forces have been well studied, much less is known about how these forces interact to influence the evolution of community structure. Here, I report an example of compensatory abiotic and biotic factors limiting the distribution of two terrestrial salamanders and effectively maintaining community structure.

The distribution of the wide-ranging *Plethodon cinereus* completely surrounds the range of *P. hubrichti,* thus it was believed that *P. hubrichti* was geographically restricted as a result of interspecific competition from *P. cinereus.* Using a combination of morphological analysis, resource use (food) data , climatic data, and ecological-niche modeling, I found no evidence to support the hypothesis that biotic interactions with *P. cinereus* have restricted the range of *P. hubrichti.* I found no partitioning of food resources in sympatry and no evidence of sympatric morphological divergence. Conversely, there was significant evidence supporting the hypothesis that abiotic forces affected the distribution of *P. hubrichti.* There was a significant association between local environmental variables and average population morphology, as well as significant morphological convergence of the two species in sympatry, both consistent with adaptation to the local environment. Additionally, ecological niche modeling accurately predicted the range of *P. hubrichti,* lending further support to this hypothesis. Most notably, even though ecological niche modeling predicted that the ecologically-viable range of *P. cinereus* included nearly 90% of the range of *P. hubrichti,* it is absent from nearly this entire region. Combined with the prior behavioral research (where *P. hubrichti* was the more aggressive of the two), these results strongly suggest that biotic interactions with *P. hubrichti* negatively impact and restrict the distribution of *P. cinereus.* This finding is the converse of what was predicted based upon the species distributions alone. This study provides a concrete example of how both biotic and abiotic forces interact to maintain joint species distributions and influence community structure. It also stresses the need for a pluralistic approach to community ecology as well as species' distributions that integrate multiple data sets.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Overview

Ecological communities exist of sets of interacting populations of different species. Abiotic, biotic, and even stochastic events are responsible for development of community structure and understanding the role of these factors in maintaining and regulating communities remains the major goal of community ecology (Gotelli 2004). More recently the burgeoning field of evolutionary ecology is being fast integrated into the machinery of contemporary community ecology. Evolutionary ecology elucidates the role of abiotic and biotic selective pressures on organisms and the evolutionary responses to such pressures. Additionally, historical contingency has also been recognized as an important factor in the development of communities (Ricklefs and Schluter 1993, Losos 1996). Thus, evolutionary community ecology maintains to examine the role of both proximate (ecological factors) and historical (evolutionary mechanisms) factors in determining community structure.

One approach to the study of community ecology is to examine continuous morphological variation within and across populations, and determine how such variation is associated with both biotic and abiotic factors (Losos 1996). Association of significant morphological variation with competitive interactions such as resource use or aggressive behavior can provide insight to the role of interspecific interactions in community maintenance and development (see Schluter and McPhail 1992, Adams and Rohlf 2000, Jaeger et al. 2002). Conversely, the relationship of morphological variation with geographic and environmental clines may suggest the role of local adaptation and environmental constraints in maintaining community structure. It is worth noting that most biologists agree that both abiotic and biotic factors govern community structure, but the relevant importance of each is not clearly understood. Researchers such as Diamond (1975) have argued that interspecific interactions clearly dominate community organization whereas others have emphasized the role of abiotic factors, such as climatic conditions, over biotic ones (see

Ricklefs and Miller 1999). Furthermore, little research has examined the role of both factors simultaneously to determine their relative role in determining community organization (however see Wilbur 1987).

For my masters' thesis I used an interdisciplinary approach to examine a two salamander community in west-central Virginia. Both salamanders are of the genus *Plethodon*, including the cosmopolitan *Plethodon cinereus* (red-backed salamander), whose range encompasses much of northeastern North America, and the Virginian endemic *Plethodon hubrichti* (Peaks of otter salamander), which has an extremely limited range. To better understand the interactions between these two species, I examined a variety of data types to determine the extent of biotic interactions and possible abiotic effects. First I quantified salamander head morphology (using landmark-based geometric morphometric methods) and examined stomach contents of a subset of collected specimens. I also incorporated knowledge of previous work on aggressive behavior of the two species (Wicknick 1995, Suprock and Highton unpublished). With this information, I examined the effects of competitive interactions on morphological variation and divergence to understand their impact to the community structure. I also studied the relationship between morphology and the environment to determine the extent of local adaptation to abiotic factors in both species. Finally, I used ecological niche modeling to determine whether abiotic environmental factors provided a reasonable prediction of current species distributions. In total, I tested three distinct hypothesis directly related to community organization and structure in this two-salamander community: (1) have biotic interspecific interactions driven significant morphological character divergence in regions of sympatry? (2) Have abiotic forces generated significant morphological convergence in regions with similar environmental pressures? (3) Are environmental conditions sufficient to explain the distribution of the species and the resultant community structure?

1.2 Thesis Organization

The content of this thesis is organized as follows. In Chapter 1 I review the biology of *Plethodon* salamanders, and describe their general life history, ecological interactions, and morphological variation. I then present a brief description of community ecology, and how both ecological and evolutionary forces can generate and maintain community structure and morphological variability. I then describe the methods used for morphological quantification (geometric morphometrics), and describe why these approaches are preferable to other alternatives. Finally, I describe methods for ecological niche modeling, and how bioclimatic data may be used to elucidate patterns of abiotic effects on ecological communities.

Chapter 2 is the primary data chapter of this thesis, and is presented as a research paper that is to be submitted to the *Proceedings of National Academy of Sciences*. This chapter provides a condensed exposition of background information and relevance of the research problem. It also summarizes the methods and analyses used in the research. All data collected and analyzed over the extent of this project to understand the geographic distribution and community structure of the two salamander species are presented in the paper. The paper examines association of morphological variation with resource use in a subset of sympatric and allopatric populations of *P. hubrichti* and *P. cinereus*; morphological association of all allopatric and sympatric populations of both species for which such data was ascertainable; and ecological-niche modeling using geographic coordinates for all known localities where species have been collected and catalogued. Finally, the discussion expounds upon what the results indicate about the role of abiotic and biotic factors in community ecology and what this portents for the direction of future research in the field. A synthesis of the results and analysis and its place in understanding the bigger picture of *Plethodon* community ecology and evolutionary community ecology in general is discussed in Chapter 3. Implications for conservation of *P. hubrichti* are also discussed. Considerations for further research and implication for conservation for *P. hubrichti* are also discussed in the final chapter.

1.3 *Plethodon* Biology and Species Description

Salamanders of the genus *Plethodon* (family: Plethodontidae) are characterized by the absence of lungs, paired premaxillae (Dunn 1926, Wake 1966), and nasolabial grooves, which connect the external nares to the cirri of the upper lip (Graves 1994). Species of the genus rank among the most abundant terrestrial vertebrates in North American forests. Current work on the systematics of the group recognizes 55 species (Highton 1962, 1972, 1995, 1999, 2005, Highton and Peabody 2000, Lazell 1998) including 46 eastern species and 9 western species. The eastern species are further subdivided in to 4 subclades that comprise of the 'large' and 'small' salamander groups. Large salamanders include the *P. glutinosus* group (29 spp.) and *P. wehrlei* (2 spp.), whereas the small salamanders include the *P. cinereus* (10 spp.) and *P. welleri* (5 spp) group. Eastern Plethodontid communities are particularly speciose around the Appalachian woodlands in north eastern North America. Up to 5 different species from eastern subclades are known to co-occur in a specific region (Highton 1995). In some areas, up to 10 different species from the family Plethodontidae can co-occur in a salamander community (Hairston 1987). Most of the eastern *Plethodon* are parapatrically distributed, with narrow zones of overlap. Additionally, some species have broad geographic ranges while others are geographically restricted. This combination of range size variation and partial overlap has produced more than 140 contact zones between eastern *Plethodon* species, providing a unique opportunity to examine species interactions in naturally-replicated ecological communities.

All species in the genus display direct development, are terrestrial throughout their life cycle, and are abundant in the woodlands of eastern and western North America. They breathe cutaneously, and are thus restricted in their activity and habitat preference by moisture and the risk of desiccation. Subsequently, they are found in moist woodlands inhabiting cover objects such as rocks and logs. They are most active in the spring and fall seasons, and after periods of rainfall they can be found actively foraging for food in the leaf litter. Their diet primarily consist of insects and other small invertebrates common in the soil and leaf litter. They are generalist predators (Jaeger 1972), and will consume any prey they encounter, provided that they are capable of ingesting it (*Plethodon* are gape-limited

predators). *Plethodon* also have many natural predators, including a *Plethodon* specialist *Diadophis punctatus* (the ringneck snake), which feeds primarily on small salamanders (Adams 1999a); several species of garter snakes (Dumas 1956, Arnold 1982); and some species of birds (Petranka 1998). *Plethodon* are long-lived for their small size, and have been known to live up to 15 years (Hairston 1987). All species examined have an elaborate courtship display as a precursor to breeding, where the male attempts to lead the female through a courtship dance (Arnold 1977). A successful courtship display results in the deposition of the male spermatophore on a substrate, which is subsequently picked up by the female with her cloaca. Small clutches of eggs (6 - 12 for *P. cinereus*) are laid in late spring and summer. Females normally remain with and guard the eggs for the 6-8 weeks until hatching.

Many years of both field and laboratory research has resulted in an intensive understanding of the biology of *Plethodon* that is crucial to our examination of community ecology of the group. First, the evolutionary relationships within the genus have been thoroughly examined. Furthermore, the ecology and behavior (including interspecific interactions) has also been well documented. Most small species have been shown to be territorial and inter-and intraspecfic competition has been demonstrated in both the field and the laboratory (e.g. Hairston 1951, 1981, Jaeger 1970, 1971a, 1972, Nishikawa 1985, 1987). Indeed the largely parapatric distribution of the species including both widely distributed species (e.g. *P. cinereus*, *P. serratus*, *P. glutionsus*) and extremely endemic species (e.g. *P. shenadoah*, *P. nettingi*, *P. hubrichti*) suggests that interspecific interactions may play an important role in determining species ranges and maintaining stable species communities. In eastern *Plethodon,* both inter- and intraspecific competition for cover objects is known to occur (Hairston 1987). In one case the highly endemic *P. shenandoah* is restricted to talus slopes of the 3 mountain peaks it inhabits by the aggressive interactions of *P. cinereus* (Jaeger 1970, 1971a). A more classical example is found between *P. glutinosus* and *P. teyahalee* in the Great Smoky Mountains. Long-term removal and transplant experiments determined that interference behavior between the species was the most likely cause of their current distribution pattern (Hairston 1951, 1973, 1980a, 1980b, 1983). This finding was confirmed via behavioral experiments performed by Nishikawa (1985, 1987), who

demonstrated greater interspecific aggression in those regions where the species had less geographic overlap. More recently evidence of character displacement via aggressive behavior was identified in this system (Adams 2004), demonstrating the important role that interference behavior plays in maintaining the community structure through evolutionary time. In other *Plethodon* communities, other forms of biotic interactions (such as exploitative competition) likely govern community interactions. For example, *P. hoffmani* and *P. cinereus* were found to compete exploitatively for food resources, and this was likely the cause of both sympatric morphological divergence and the maintenance of the *Plethodon* community (Adams 2000, Adams and Rohlf 2000). Subsequent behavioral experiments ruled out the alternative, that behavioral interference had driven morphological change, followed by food partitioning (Jaeger et al. 2002).

While the role of biotic interactions have been thoroughly examined in *Plethodon* communities, abiotic and bioclimatic forces have been much less examined. As with most terrestrial ectotherms, climatic conditions must significantly limit species distributions, and therefore their fundamental niche. In particular, moisture levels are likely an important factor in limiting distributions of species in the group, because of the propensity of desiccation during dry spells. In fact, moisture may well be a limiting factor that alters behavioral patterns in *Plethodon* (Jaeger 1971b). Physiological restrictions within certain groups, along with interspecific competition, may also be important in maintaining contact zones and stable communities. For instance the more aggressive *P. cinereus* is physiologically restricted in completely engulfing the range of the endangered *P. shenandoah*, owing to the latter's inhabitation of relatively drier talus slopes which is not an amenable habitat for *P. cinereus* (Jaeger 1970, 1972). Based upon such evidence, one may therefore hypothesize that there may be an association between morphological variation and environmental parameters for species in some communities

In this study, I examine communities with two primary species: *P. cinereus* and *P. hubrichti*. Both are members of the small *P. cinereus* group, and are thus closely related. *P. cinereus* is a cosmopolitan species with a range extending through most of the northeastern United States and southeastern Canada (Petranka 1998). Conversely, *P. hubrichti* is an extremely range-restricted species found in west-central Virginia. Its entire range is

approximately 45 square miles overlapping the boundaries of Bedford and Boutetourt counties in the vicinity of Jefferson National Park and the Blue Ridge Parkway (Pague and Mitchell 1990). Two known sites of sympatry between the species occur. The large *P. glutinosus* also coexists in the sympatric zones, but is uncommon in those areas (personal observation).

Through much of its range, *Plethodon cinereus* inhabits cool, moist coniferous, mixed, and hardwood forests from sea level to 1707 m (Petranka 1998 and references therein). Population densities of the species range from 0.05/ m^2 (Test and Bingham 1948) to 2.2 /m^2 (Jaeger 1980). The species is quite aggressive and highly territorial (Gergits 1982, Jaeger 1970, 1979). In the Blue Ridge Mountains, the range of *P. cinereus* completely surrounds that of *P. hubrichti. Plethodon hubrichti* is endemic to the Peaks of Otter area in Virginia and can be found in mature hardwood forests under cover objects (Mitchell 1991). Its surface population density varies from 0.24/m^2 to an estimated density of 4.5/m^2 (Kramer et al. 1993). It is restricted to elevations above 550m (Mitchell 1991), and its average adult snout-vent length is usually larger than that of adults of *P. cinereus*.

Interestingly, despite the restricted range, previous laboratory experiments of competitive interactions between the two species have shown *P. hubrichti* to be more aggressive and more successful in defending territories against *P. cinereus* (Wicknick 1995). In a field study, transplant and removal experiments showed *P. hubrichti* to have a negative effect on *P. cinereus*, but the converse effect of *P. cinereus* on *P. hubrichti* was not found (Suprock and Highton unpubl.) Both species are also larger in body size in the sympatry relative to allopatry (Wicknick 1995). These results run counter to the expectation that *P. hubrichti* is geographically restricted as a result of interspecific competition from *P. cinereus* (e.g., Highton 1972, Jaeger 1970, 1971a, 1974, Nishikawa 1985, 1987). Hence, the restriction of *P. hubrichti's* range in the light of it being a more aggressive species remains a mystery. Because the forces that govern the distributions of these 2 species remain elusive, I felt it necessary to examine this ecological system from a more comprehensive perspective.

For this study, I brought to bear knowledge of the behavioral interactions from previous studies (Wicknick 1995) between the species of *P. cinereus* and *P. hubrichti*, could be easily exploited in hypothesis testing of a model of competition (at least qualitatively) which

examines the role of interference competition between the species in maintaining a stable community. Acquisition of 446 museum catalogued specimens also helped in collecting an intensive amount of data on morphology of species from sympatric and allopatric sites. The morphological data obtained from these and collected specimens was used extensively for testing our hypothesis on the role of both abiotic and biotic factors in governing community structure in sympatric sites of the two species. The particular geographic distribution, specimen availability, and a wealth of background knowledge on *Plethodon* species facilitate them to be a naturally replicated experiment in the study evolutionary community ecology. This dual perspective approach to elucidate the community structure of *P. hubrichti* and *P. cinereus* in this research represents one such replicate in the larger body work of that seeks to determine the rules governing *Plethodon* community ecology in general.

1.4 Community Structure, Interspecific Interactions, and Evolutionary Change

Community structure relates to the patterns of species abundance and the various interaction of populations within that community (Ricklefs and Miller 1999). As such, the organization and maintenance of community structure is the result of a suite of biotic, abiotic, and even stochastic events, as well as interactions between these factors. This vast array of interactions, directly and indirectly, ties all members of a community together into a complex and multifaceted network. Populations of individual species extend their influence onto other members of the community through prevalence of biotic interactions such as competition and predator/prey relationships (Ricklefs and Miller 1999). The importance of biotic interactions and their influence on community structure has been long established, and is reviewed elsewhere (Gurevitch et al. 1992, 2000). Some ecologists have even argued that interspecific interactions are the major force driving community structure (Diamond 1975). The existence of competition and predation individually as dominant forces in community structure has been empirically demonstrated in many ecological communities (for predation examples see

Sih et al. 1998, Bertness et al. 2004; for competition examples see Melville 2002, Almany 2003, see also Schluter 2000).

Though the physical environment greatly influences community characteristics, if resources are limiting then competition can also play a large role (perhaps best demonstrated by the laboratory experiments of Gause (1934). Competition between species can lead to two likely results: (i) competitive exclusion of 1 species or (ii) coexistence of both species through a mechanism such as character displacement. The competitive exclusion principle (Hardin 1960) dictates that two ecologically similar species cannot coexist when both species rely on the same 'limiting' resource. Eventually one species will be driven to extinction or at least excluded from the range of the species that utilizes the limiting resource more effectively. However, in nature, similar species do tend to coexist. Such cases have been inferred to result when two competing species reduce competition among themselves through niche partitioning. Such niche partitioning is concomitant with divergence of some character previously similar between the two species. This divergence may be in behavior, morphology, and/or physiology and is termed character displacement. Note character displacement occurs over evolutionary time.

One popular approach to inferring the role of competition in community structure is to examine species distributions across sets of communities, and determine whether species coexist less frequently than is expected by chance (e.g., Diamond 1975, Weiher et al. 1998, Brown et al. 2002). When such 'checkerboard' patterns are found, this non-random species co-occurrence pattern is attributed to interspecific competition, where such communities consist of ecologically differentiated taxa build up over evolutionary time leaving patterns as the ghost of competition past. This particular flavor of community ecology seeks to find results that are concordant with the competitive exclusion principle, where competing species co-occur less frequently than by chance. Initially, such research (Diamond 1975) was met with controversy (e.g., Connor and Simberloff 1979), though a recent meta-analysis (Gotelli and McCabe 2002) suggests that these methods have positively identified an ecological pattern consistent with interspecific competition.

Another approach examines the role of interspecific competition in communities of

ecologically similar species.. Competition theory predicts that two ecologically similar species may coexist only through some form of niche partitioning for a limiting resource and this is followed by divergence in some character associated with utilizing the particular resource (Brown and Wilson 1956, Hutchinson 1959). The prediction of character displacement can be tested in communities of coexisting species if the species also occur individually in allopatry. Based on these predictions this second approach looks to test whether two coexisting species are significantly more divergent in a particular character(s) in sympatry as compared with their allopatric conspecifics (see Schluter and McPhail 1992, Adams and Rohlf 2000). The character(s) in question may be morphological, physiological, and/or behavioral, and are often associated with the limiting resource (such as food in many cases). While the prevalence of character displacement has been disputed over the years, recent evaluations suggest that it is quite prevalent in nature (Schluter 2000).

Alternatively, abiotic selective pressures can be predominant in influencing character divergence in communities. However, when character change is brought upon by the physical environment it is hypothesized to be convergent rather than divergent since all species in the community will be exposed more or less to similar abiotic pressures. Character convergence relates to the event of two species becoming significantly more similar or convergent in a particular character(s) in sympatry as opposed to their conspecifics in allopatry. Convergence of characters, then intuitively is more likely the result of ecologically-driven selective pressures, such as abiotic environmental constraints (see Arthur 1982, Webb at al. 2002). Conversely, species that fail to adapt to the similar environmental constraints may also go extinct (Web et al. 2002). Communities that consist of species that are convergent in a particular character(s) are said to have undergone ecological or habitat filtering.

Within *Plethodon* communities, competition has been long believed to be an important determinant of community structure (predation among congeneric species is rare). Additionally, the influence of both interference and exploitative competition has been demonstrated in multiple *Plethodon* communities (Hairston, 1951; 1973; 1980a, 1980b, 1983, Jaeger 1970, 1971, Adams 1999; 2004, Adams and Rohlf 2000). Our background knowledge on the interspecific interactions and the distributions of focal species entails a specific list of possible hypothesis. We know our focal species display aggressive behavior

towards each other (Wicknick 1995.) This competition or perhaps presence of exploitative competition may be detectable in the form of character displacement (See Adams and Rohlf 2000, Jaeger et al. 2002). This would suffice in determining competition as the major determinant of the community structure of the species. Conversely, vegetation surveys and topography of the region (Wicknick 1995) around *P. hubrichti's* native range delineates no drastic change in environmental conditions. Therefore, allopatric *P. cinereus* in the neighboring area do not occupy drastically different environmental regimes. Thus, sympatric localities can be said to be occurring along ecotones, in this case no significant divergence in morphology may be detected and the structure of the community may be attributed to absolute range limitations. Alternatively, sympatric congenerics may be found to be significantly morphologically more similar as compared to allopatric conspecifics. This result would suggest that perhaps some fine-grained environmental constraints (such as some ecological or physiological attribute that limits *P. hubrichti* to be found only 550m above sea-level) determine structure and stability of this community.

1.5 Geometric Morphometrics

1.5.1 Importance and Emergence of Geometric Morphometrics

Quantification of biological form has been an active research area for over one hundred years. Quantitative morphology has long been used to assess ecological and evolutionary hypotheses, because the size and shape of an organism are intimately related to organism identification, classification and taxonomy, biodiversity, and functional morphology. Therefore, quantifying morphological variation within and between species allows one to better understand the relationships between the ecology, evolution, and morphology (Losos 1990, Loy et al. 1996). Bumpus's work on sparrows (1898) is an early example of quantifying morphology to test biological hypothesis. The field of quantitative morphology is called "Morphometrics", and is described as the study of shape variation and its covariation with other variables (Bookstein 1991).

However, while morphology has been quantified and used in biological research for centuries, morphometrics only came of age in the mid-twentieth century. Not surprisingly, advances in morphometric methodology developed in concert with the development of rigorous statistical methods; such as correlation coefficient, analysis of variance, and Principal components. During much of this time, the most frequently used morphological variables were linear measurements such as length, width, and height. The use of multivariate statistics on sets of linear distance methods became known as traditional morphometrics, or multivariate morphometrics (Reyment 1991). However, while these methods proved useful for identifying patterns of phenotypic differences among groups of biological objects, they also had several shortcomings that limited their utility (see Rohlf and Marcus 1993 for a review). One such difficulty arose from the fact that linear measurements themselves contain not only shape information but also size information. To remove the effect of size from linear distances, many 'size-correction' approaches have been developed (for a review see Jungers et al 1995). Unfortunately, there exists no objective criterion for preferring one method over the other as none of the methods are mathematically equivalent. Hence, statistical results can differ depending on which size-correction method is chosen. Another important shortcoming of using linear measurements in shape analysis lays in the inadequacy of such measurements to retain the geometry of the original shape. This impacts the analysis of shape in two ways. First, the graphical representation of shape is not possible with linear measurements, so one cannot describe *how* shape differs (Rohlf 1999). Second, the geometric components of shape are not captured with linear distances (because the relationships between distances are lost), and so this important aspect of shape and shape variation is not statistically examined. As a result, shape differences between objects are not detected with linear distances, demonstrating that these approaches provide an incomplete picture of shape variation.

An alternative approach which is more widely used in recent years (see Adams et al. 2004), is geometric morphometrics. With this approach, morphology is quantified using the x,y (or x,y,z) coordinates of biologically repeatable or homologous landmarks. Non-shape information in these coordinates is then removed, and the size, and a set of shape variables are obtained. Traditional multivariate statistics can then be used on these shape variables for hypotheses testing. The application and development of geometric morphometrics has

exponentially increased since their advent in the late 1980' s and continues to promise further advances in analysis of shape, as well as for various specialized applications for particular biological hypotheses (e.g., motion analysis, asymmetry, quantitative genetics, etc.: see Adams et al. 2004 for a review of the role of geometric morphometrics in the last two decades). Indeed, the development of geometric morphometrics has been likened to a 'revolution' (Rohlf and Marcus 1993) in the sense of a true paradigm shift in this field. This paradigm shift occurred through the realization that one can preserve the geometric aspects of shape by simply utilizing a new data type, and thereby increase the predictive power of analyses of shape variation and its covariation with other variables. While geometric morphometric methods for both landmark/point and outline data have been developed (see Bookstein 1997 for information on outline analysis), in my research, I quantified morphology using landmark-based geometric methods. The general approach is described below.

1.5.2 Acquiring Shape Data Using Geometric Morphometrics

All landmark-based geometric morphometric methods begin by acquiring the two- or three- dimensional coordinates of homologous and biologically-definable points (landmarks) across all specimens. The choice of appropriate landmarks is directly related to the kind hypothesis the researcher wishes to test. Once the landmark coordinates are obtained, the next step is to remove any non-shape variation, which includes variation in the size of specimens, and their position and orientation at the time of digitization. This non-shape variation is removed using a generalized least-squares (Procrustes) superimposition (Generalized Procrustes Analysis: GPA, Rohlf and Slice 1990). Using GPA, one translates all specimens to a common location, scales them to a unit size, and rotates them until the sum of squared differences of corresponding landmarks between a specimen and a reference configuration is minimized. Procrustes superimposition can be mathematically represented as:

$$X_i = \rho XH + 1\tau$$

where ρ represents scaling the object to centroid size, 1τ is the vector that translates the centroid of the origin, H is the rotation matrix that best aligns X_i to X in a least-squares sense, and X is the original p x k configuration of p landmarks in k dimensions (see Slice 1996). For

more than two specimens, this approach is generalized (iterated) such that one first rotates all objects to the first object in the data set, calculates an average configuration, and then aligns all specimens to this consensus (Rohlf and Slice 1990). This procedure is iterated until the total error sums of squares is minimized and converges.

After GPA superimposition, shape variation can be described using the resultant residual coordinates. However, because the specimens were standardized for position, orientation and scale, these values are not independent, and their covariance matrix is singular. Therefore, multivariate statistics that utilize the inverse of the covariance matrix, such as MANOVA, CVA, etc. cannot be performed on this data. To remove these redundant dimensions, the thin-plate spine (TPS) is used (Bookstein 1991). TPS projects each object into a linear tangent space, and maps the change or deformation in shape from one specimen to another using a smooth interpolating function. Through projection, the redundant dimensions are removed. Additionally, shape is now represented in a linear tangent space (Rohlf 1999), so coordinates of the axes of this space may serve as shape variables, and can be used in standard multivariate statistical techniques to examine shape variation within and among groups (Rohlf 1999).

An additional benefit of TPS is that this interpolating function can be used to generate graphical depictions of shape for visual interpretation. Using this approach, the coordinates of one specimen are mapped to those of another, describing the difference in shape between the two specimens in terms of deformation grids. The shape on one specimen as the deformation of another can be comprehensively described by the parameters of the TPS function that includes a uniform and non-uniform component (Bookstein 1991). For two dimensional data, there are two uniform shape variables describing affine shape variation (Bookstein 1996) and 2p-6 non-uniform shape variables describing non-affine variation. The non-uniform variables can be further decomposed into orthogonal elements called partial warps. The partial warp scores and the uniform components describe affine shape variation taken together can be effectively used as shape variables for hypothesis testing.

Using these variables generated from TPS we can describe shape variation between and within groups. The same shape variables can also be used as input to the suite of extant multivariate statistic methods including but not restricted to PCA, MANOVA, multivariate

regression, CVA. Relationship between shape and any other variable(s) of choice (for e.g. food use, climatic) variables can also be examined with extant statistical tools. Furthermore, the deformation grids obtained from the TPS can be used to visually examine shape differences. Finally, because standard geometric morphometric analyses consist entirely of linear transformations (GPA, TPS, and multivariate statistics), one can work backwards from any point to generate a graphical representation of the phenotypic space of the organism (Adams 1999). Hence, we can not only statistically confirm the presence of some shape pattern but also visually discern it through deformation grids.

1.5.3 A Comparison to Other Landmark-Based Shape Analysis

Several other landmark-based morphometrics approaches have been developed, which include Euclidean distance matrix analysis (EDMA), finite element scales analysis (FESA), and several methods based on interior angles. However, GPA based analysis of shape variation has been highly advocated based on several drawbacks of these other methods (Adams et al. 2004). First, the statistical power (or ability to discern true differences in means) for methods based on interlandmark distances and angles has found to be much lower and never higher than GPA methods in many different scenarios (Rohlf 2000a). The study also showed that GPA methods had the best Type I error rates (rejecting a null hypothesis when it is true) among the other methods. Another simulation study (Rohlf 2000b) also showed that in ordination analysis such non-GPA methods exhibited distinctive patterns of covariation within and between groups that was dependent on their average shapes. Such patterns (where random data induces some distinctive pattern) would mislead the investigator about the true shape variation among her organisms. A more recent simulation study compared Mean Square Error (MSE) and Bias among the different methods to determine which method most closely estimates to the true mean value. The magnitude and pattern of any bias to the mean estimates from these methods was also examined. GPA followed by projection into tangent space was the only method of landmark-based shape analysis that had a low MSE and no pattern of bias. The ability to not induce any pattern in data, high statistical power, reliable Type I error rates, low MSE and no pattern of bias,

provide any researcher of shape analysis with an objective criterion to prefer GPA landmark based approaches over other methods.

1.6 Species Distribution and Ecological-Niche Modeling

1.6.1 Abiotic Factors and Species Distributions

The Gleasonian concept of ecological communities (Gleason 1926) describes communities as resulting from the fortuitous overlap of individual species distributions. This 'open view' of communities has been an important component of community ecology since its introduction. Until the 1960's, the opposing view of closed communities (Clements 1936) had been a formidable opponent to the theory of open communities. However, initial work with gradient analysis, particularly that by Robert Whittaker (1960), was highly effective in determining that the Clementsian view of community ecology was less supported by the data.

Gradient analysis is procedure that maps species abundance along a particular cline ecological cline (Loucks 1962). A related procedure developed to describe community structure is ordination (Loucks 1962, Ricklefs and Miller 1999). The purpose of ordination is to generate an n-dimensional space describing relationship between environmental variables and then arranging communities within this data space. Information obtained from research incorporating gradient analysis and ordination analysis did much to advance the continuum concept of community organization, which is a logical articulation of the open community, such that species in communities are gradually replaced by others when their range of ecological and physical limitations is reached. For closely related taxa, many communities consist of secondary contact zones that often occur at ecotones (Case and Taper 2000). Ecotones are defined as transitions of community type, particularly in terms of change in biotic vegetation, soil types and environmental conditions. Therefore, it is widely acknowledged that many species communities, particularly ones of closely related taxa, are secondary contact zones that have resulted from some magnitude of overlap of the individual species distribution. It is also widely recognized that absolute distributions of species, particularly of ectotherms, are governed by climatic regimes.

1.6.2 Ecological-Niche Modeling

The advent of geographic information systems (GIS) and ecological-niche modeling Using BIOCLIM (Nix 1986) and GARP (genetic algorithm for rule-set production) analysis (Stockwell and Peters 1999) have provided us with the opportunity of predicting broad-scale species distributions in relation to environmental variables (Peterson 2001). Such ecological-niche modeling takes a set of geo-referenced localities where a particular species is present and then correlates it to corresponding environmental data to predict the possible extent of the species distribution based on that data. It can not only be used to determine what environmental variables are integral in determining particular species distributions but also predicts other areas are suitable for habitation by the species. These models can have been successfully applied to plethora of questions regarding species distributions in terms of ecological and evolutionary phenomena (Cicero 2004, Nix 1986, Peterson et al. 2001, Raxworthy et al. 2003, Ricthie et al. 2001). Additionally, combining morphological and or molecular data with ecological-niche modeling (Cicero 2004) can be used to test alternative hypotheses of the forces governing species ranges, such as gene flow versus local adaptation (Case and Taper 2000).

In this thesis, I used the BIOLCIM algorithm (Nix 1986) incorporated within the DIVA-GIS program (Hijmans et al. 2004) to predict possible distributions for both focal species. BIOCLIM is one of the simplest ecological-niche modeling procedures available and has been used extensively in predicting species distributions (Peterson 2001). Incorporation of the BIOCLIM algorithm in DIVA-GIS has immensely eased the procedure of running bioclimatic models on desktop computers. Apart from the input of species localities (as points) the BIOCLIM algorithm extracts 19 derived bioclimatic variables across a 1-km-resolution grid points (lower resolutions are available). The best available resolution of bioclimatic data was used to enhance accuracy of the algorithm (Nix 1986). BIOLCIM data are parameters derived from mean monthly climate estimates, and the parameters are calculated to approximate energy and water balances at a particular location (Nix 1986). Parameters BC1-19 were used which include: annual mean temperature, mean diurnal range(Mean(period max-min)), isothermality, temperature seasonality (standard deviation *100), max temperature of warmest period, min temperature of coldest period, temperature

annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest period, precipitation of driest period, precipitation seasonality(coefficient of variation), precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter. All available indices were used to prevent a potential over-prediction of species distributions.

Running the BIOLCIM algorithm data consist of several simple steps before a species prediction map is developed. Firstly the algorithm categorically counts the species occurrences for each individual BIOCLIM variable. It then creates a distribution for each variable truncating the extreme edge of distributions as specified by the user (percentile cut-offs for distribution). An aggregation of the truncated distributions is then calculated to provide a decision rule to predict all possible ecological viable areas inhabitable by a species within a specified region.

1.7 Literature Cited

Adams, D. C. 2004. Character Displacement via Aggressive Interference in Southern Appalachian Mountain Salamanders. *Ecology* 85:2264-2670.

Adams, D. C. 2000. Divergence of trophic morphology and resource use among populations of *Plethodon cinereus* and *P. hoffmani* in Pennsylvania: a possible case of character displacement. Pp. 383-394 in *The Biology of Plethodontid Salamanders.* (R. C. Bruce, R. J. Jaeger, and L. D. Houck, eds.). Klewer Academic/Plenum. New York.

Adams, D.C. 1999. *Plethodon hoffmani*. Predation *Herpetological Review.* 30:160.

Adams, D.C. 1999. Ecological character displacement in *Plethodon* and methods for shape analysis of articulated structures. Ph.D. Dissertation. State University of New York at Stony Brook, Stony Brook, New York.

Adams, D. C., and F. J. Rohlf. 2000. Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences, U.S.A.* 97:4106-4111.

Adams, D. C., F. J. Rohlf, and D. E. Slice. 2004. Geometric morphometrics: Ten years of progress following the 'revolution.' *Italian Journal of Zoology.* 71:5-16.

Almany G.R. 2003. Priority effects in coral reef fish communities. *Ecology*. 84:1920-1935.

Arnold, S.J. 1982. A quantitative approach to antipredator performance: salamander defense against snake attack. *Copeia.* 1982:247-253.

Arnold, S.J. 1977. The evolution of courtship behavior in New Wolrd salamanders with some comments on Old World salamandrids. In *The reproductive biology of amphibians.* (Taylor, D.H., and S.I. Guttman, eds.). Pp. 141-183. Plenum Press, New York.

Arthur, W.A. 1982. The evolutionary consequences of interspecific competition. *Advances in Ecological Research.* 12:127-187.

Bertness M.D., G. C. Trussell, P. J. Ewanchuk, B. R. Silliman, and C. M. Crain 2004. Consumer-controlled community states on Gulf of Maine rocky shores. *Ecology.* 85:1321-1331.

Bookstein, F. L. 1991. *Morphometric tools for landmark data: geometry and biology.* Cambridge University Press, Cambridge.

Bookstein, F. L. 1996. A standard formula for the uniform shape component in landmark data. In *Advances in morphometrics*. (Marcus, L.F., M. Corti, A. Loy, G. Naylor, and D. Slice, eds.). Pp. 153-168. Plenum Press, New York.

Bookstein, F.L. 1997. Landmark methods for forms without landmarks: localizing group differences in outline shape. *Medical Image Analysis.* 1:225-243.

Brown, J. H., D. A. Kelt, B. J. Fox. 2002. Assembly rules and competition in desert rodents. *American Naturalist.* 160:815-818.

Brown, W. L., and E. O. Wilson. 1956. Character displacement. *Systematic Zoology.* 5:49-64.

Bumpus, H.C. 1898. The elimination of the unfit as illustrated by the introduced sparrow *Passer domesticus.* Biological Lectures, Marine Biological Laboratory, Woods Hole 11:209-226.

Case, T. J., and M. L. Taper. 2000. Interspecific competition, environmental gradients, gene flow, and the coevolution of species borders. *American Naturalist.* 155:583–605.

Cicero, C. 2004. Barriers to sympatry between avian sibling species (Paridae: *Baelophus*) in local secondary contact. *Evo.lution* 58:1573-1587.

Clements, F.E. 1936. Nature and the structure of climax. *Journal of Ecology.* 24:252-284.

Connor, E. F., and D. Simberloff. 1979. The assembly of species communities: chance or competition? *Ecology.* 60:1132-1140.

Diamond, J. M. 1975. Assembly of species communities. Pages 316-331 *in* M. L. Cody and J. M. Diamond, eds. *Ecology and Evolution of Communites*. Belknap Press of Harvard University Press, Cambridge.

Dumas, P.C. 1956. The ecological relations of sympatry in *Plethodon dunni* and *Plethodon vehiculum*. *Ecology.* 37:484-495.

Dunn, E.R. 1926. *Salamanders of the family Plethodontidae.* Smith College, Northampton.

Gause, G.F. 1934. *The Struggle for Existence*. Williams and Wilkins, Baltimore.

Gergits, W.F. 1982. Interference competition and territoriality between the terrestrial salamanders *Plethodon cinereus* and *Plethodon shenandoah*. M.S. Thesis, State University of New York at Albany, Albany, New York.

Gleason, H.A. 1926. The individualistic concept of the plant association. *Torrey Botanical Club Bulletin.* 53:7-26.

Gotelli, N. 2004. A taxonomic wish-list for community ecology. *Philosophical Transactions of the Royal Society of London, Series B.* 359:585-597.

Gotelli, N., and D. J. McCabe. 2002. Species co-occurrence: a meta-analysis of J. M. Diamond's assembly rules model. *Ecology.* 83:2091-2096.

Graves, B.M. 1994. The role of nasolabial grooves and the vomeronasal system in recognition of the home area by red-backed salamanders. *Animal Behavior.* 47:1216-1219.

Gurevitch, J., L. L. Morrow, A. Walsh, and J. S. Walsh. 1992. A meta-analysis of competition in field experiments. American Naturalist. 140:539-572.

Gurevitch, J., J. A. Morrison, and L. V. Hedges. 2000. The interaction between competition and predation: a meta-analysis of field experiments. American Naturalist. 155:435-453.

Hairston, N. G. 1951. Interspecies competition and its probable influence upon the vertical distribution of Appalachian salamanders of the genus *Plethodon. Ecology.* 32:266-274.

Hairston, N. G. 1973. Ecology, selection and systematics. *Brevoria.* 414:1-21.

Hairston, N. G. 1980a. The experimental test of an analysis of field distributions: Competition in terrestrial salamanders. Ecology. 6:817-826.

Hairston, N. G. 1980b. Evolution under interspecific competition: Field experiments on terrestrial salamanders. Evolution. 34:409-420.

Hairson, N. G. 1981. An experimental test of a guild: salamander competition. *Ecology.* 62:65-72.

Hairston, N. G. 1983. Alpha selection in competing salamanders: Experimental verification of an a priori hypothesis. *American Naturalist.* 122:105-113.

Hairston, N. G. 1987. *Community ecology and salamander guilds.* Cambridge University Press, Cambridge.

Hardin, G. 1960. The competitive exclusion principle. *Science.* 131:1292-1297.

Highton, R. 1962. Revision of North American salamanders of the genus *Plethodon. Bulletin of the Florida State Museum* 6:235-367.

Highton, R. 1972. Distributional interactions among eastern North American salamanders of the genus *Plethodon*. In *The distributional history of the biota of the southern Appalachians. Part III: vertebrates.* (Holt, P.C., ed.). Pp 139-188. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Highton, R. 1995. Speciation in eastern North American salamanders of the genus *Plethodon. Annual Review of Ecology and Systematics.* 26:579-600.

Highton, R. 1999. Geographic protein variation and speciation in the salamanders of the *Plethodon cinereus* group with the description of two new species. *Herpetologica.* 55:43-90.

Highton, R. 2005. A new species of woodland salamander of the *Plethodon cinereus* group from the Blue Ridge mountains of Virginia. *Jeffersonia*, Number 14, pp. 1-22. Virginia Museum of Natural History, VA.

Highton, R., and R. B. Peabody. 2000. Geographic protein variation and speciation in salamanders of the *Plethodon jordani* and *Plethodon glutinosus* complexes in the southern Appalachian Mountains with the descriptions of four new species. Pp. 31-93 in *The biology of Plethodontid salamanders.* (R. C. Bruce, R. J. Jaeger, and L. D. Houck, eds.). Klewer Academic/Plenum. New York.

Hijmans, R. J., L. Guarino, C. Bussink, P. Mathur, M. Cruz, I. Barrentes, and E. Rojas. 2004. *DIVA-GIS. Ver. 5.0.* A geographic information system for the analysis of speciesdistribution data. Manual available at http://www.diva-gis.org.

Hutchinson, G. E. 1959. Homage to Santa Rosalia, or Why are there so many kinds of animals? *American Naturalist.* 93: 145-159.

Jaeger, R. G. 1970. Potential extinction through competition between two species of terrestrial salamanders. *Evolution.* 24:632-642.

Jaeger, R.G. 1971a. Competitive exclusion as a factor influencing the distribution of two species of terrestrial salamanders. *Ecology.* 52:632-637

Jaeger, R.G. 1971b. Moisture as a factor influencing the distribution of two species of terrestrial salamanders. *Oecologia.* 6:191-207.

Jaeger, R. G. 1972. Food as a limited resource in competition between two species of terrestrial salamanders. *Ecology.* 53:535-546.

Jaeger, R.G. 1974. Competitive exclusion: comments on survival and extinction of species. *Bioscience.* 24:33-39.

Jaeger, R.G. 1979. Seasonal spatial distributions of the terrestrial salamander *Plethodon cinereus*. *Herpetologica.* 35:90-93.

Jaeger, R. G., E. D. Prosen, and D. C. Adams. 2002. Character displacement and aggression in two species of terrestrial salamanders. *Copeia.* 2002:391-401.

Jungers, W.L., A.B. Falsetti, and C.E. Wall. 1995. Shape, relative size, and size-adjustments in morphometrics. *Yearbook of Physical Anthropology.* 38:137-161.

Kramer, P., N. Reichenbach, M. Hayslett, and P. Sattler. 1993. Population dynamics and conservationg of the Peaks of Otter salamander, *Plethodon hubrichti. Journal of Herpetology.* 27:431-435.

Lazell, J. 1998. New salamander of the genus *Plethodon* from Mississippi. *Copeia.* 1998:967-970.

Losos J. B. 1996. Phylogenetic perspectives on community ecology. *Ecology.* 77: 1344-1354.

Losos, J. B. 1990. A phylogenetic analysis of character displacement in Caribbean *Anolis* lizards. *Evolution.* 44:558-569.

Loucks, O.L. 1962. Ordinating forest communities by means of environmental scalars and phytosociological indices. *Ecological Monographs.* 32:137-166.

Loy, A., S. Cataudella, and M. Corti. 1996. Shape changes during the growth of the sea bass, *Dicentrarchus labrax* (Teleostea: Perciformes), in relation to different rearing conditions: an application of thin-plate spline regression analysis. In *Advances in Morphometrics.* (L.F. Marcus, M. Corti, A. Loy, G. Naylor, and D. Slice, eds.). Pp 399-405. Plenum Press, New York.

Mitchell, J.C. 1991. Amphibians and reptiles. Pp 411-476. In *Virginia's Endangered Species* (K. Terwilliger, coordinator). Macdonald and Woodard Publishing Company, Blacksburg, Virginia.

Nishikawa, K. C. 1987. Interspecific aggressive behaviour in salamanders: species-specific interference or misidentification? *Animal Behaviour.* 35:263-270

Nishikawa, K. C. 1985. Competition and the evolution of aggressive behavior in two species of terrestrial salamanders. *Evolution.* 39:1282-1294.

Nix, H. 1986. A biogeographic analysis of Australian elapid snakes. In*Atlas of elapid snakes of Australia.*(R. Longmore, ed.) . Pp. 4-15. Australian Government Publishing Service, Canberra.

Pague, C.A. and J.C. Mitchell. 1990. The distribution of the Peaks of Otter salamander (*Plethodon hubrichti*). Unpublished report submitted to Jefferson National Forest by the Virginia Department of Conservation and Recreation, Division of Natural Heritage.

Peterson, A. T. 2001. Predicting species' geographic distributions based on ecological niche modeling. *Condor.* 103:599–605.

Peterson, A. T., V. Sa´nchez-Cordero, J. Sobero´n, J. Bartley, R. W. Buddemeier, and A. G. Navarro-Siguenza. 2001. Effects of global climate change on geographic distributions of Mexican Cracidae. *Ecological Modeling.* 144:21–30.

Petranka, J.W. 1998. *Salamanders of the United States and Canada.* Smithsonian Institution Press, Washington D.C.

Raxworthy, C.J., E. Martinez-Meyer, N. Horning, R.A. Nussbaum, G.E. Schneider, M.A. Ortega-Huerta, and A.T. Peterson. 2003. Predicting distributions of known and unknown reptile species in Madagascar. *Nature.* 426:837-841.

Reyment, R.A. 1991. *Multidimensional Pale biology.* Pergamon Press, New York.

Ricklefs, R.E., and G.L. Miller. 1999. *Ecolo*gy. 4th ed. W.H. Freeman and Company, New York, New York, U.S.A.

Ricklefs, R. E., and D. Schluter, eds. 1993. *Species diversity in ecological communities: historical and geographical perspectives.* University of Chicago Press, Chicago.

Ritchie, M. G., D. M. Kidd, and J. M. Gleason. 2001. Mitochondrial DNA variation and GIS analysis confirm a secondary origin of geographical variation in the bushcricket *Ephippiger ephippiger* (Orthoptera: Tettigonioidea), and resurrect two subspecies. *Molecular Ecology.* 10:603–611.

Rohlf, F.J. 2000a. Statistical power comparisons among alternative morphometric methods. *American Journal of Physical Anthropology* 111:463-478.

Rohlf, F.J. 2000b. On the use of shape spaces to compare morphometric methods. *Hystrix.* 11:9-25.

Rohlf, F. J. 1999. Shape statistics: Procrustes superimposition and tangent spaces. *Journal of Classification.* 16:197-223.

Rohlf, F.J., and Marcus, L.F. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution* 8:129-132.

Rohlf, F. J., and Slice, D. E. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*. 39:40-59.

Schluter, D. 2000. Ecological character displacement in adaptive radiation. *American Naturalist*. 156:S4-16.

Schluter, D., and J. D. Mcphail. 1992. Ecological character displacement and speciation in sticklebacks. *American Naturalist*. 140:85-108.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution*. 13:350-355.

Slice, D.E. 1996. Three-dimensional, generalized resistant fitting and the comparison of least-squares and resistant-fits. In *Advances in Morphometrics*. (L.F. Marcus, M. Corti, A. Loy, G. Naylor, and D. Slice, eds.). Pp 399-405. Plenum Press, New York.

Stockwell, D. R. B., and D. P. Peters. 1999. The GARP modeling system: Problems and solutions to automated spatial prediction. *Intelligence Journal of Geographic Information Systems*. 13:143-158.

Suprock, G.H., and R. Highton. Unpublished. Ecological interactions of *Plethodon* in the Blue Ridge of Virginia. Ph.D. Dissertation. University of Maryland, College Park, Maryland.

Test, F.H. and B.A. Bingham. 1948. Census of the a population of redbacked salamander, *Plethodon cinereus*. *American Midlands Naturalist*. 39:362-372.

Wake, D.B. 1966. Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. *Memoirs of the Southern California Academy of Sciences*. 4:1-111.

Weiher E, G. D. P. Clarke, and P. A. Keddy. 1998. Community assembly rules, morphological dispersion, and the coexistence of plant species. *Oikos*. 81:309-322.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics*. 33:475-505.

Whittaker, R.H. 1960. Vegetation of the Siskiyou mountains, Oregon, and California. *Ecological Monographs*. 26:1-80.

Wicknick, J. A. 1995. Interspecific competition and territoriality between a widespread species of salamander and a species with a limited range. Ph.D. Dissertation. University of Southwestern Louisiana, Lafayette, LA.

Wilbur, H.M. 1987. Regulation of Structure in Complex Systems: Experimental Temporary Pond Communities. *Ecology*. 68: 1437:1452.

CHAPTER 2: NICHE-MODELING, MORPHOLOGY, AND BEHAVIOR REVEAL COMPENSATORY MECHANISMS REGULATING THE DISTRIBUTIONS OF TWO INTERACTING SALAMANDERS

A paper to be submitted to the *Proceedings of the National Academy of Sciences*

Dean C. Adams[1], Saad Arif[2], and Jill A. Wicknick[3]

Abstract

A goal of evolutionary ecology is to understand the forces that generate ecological communities and maintain species boundaries. Although the effects of biotic and abiotic forces have been well studied, little is known about how these forces interact to influence community structure. Here we report an example of compensatory biotic and abiotic forces regulating the distributions of two salamander species. *Plethodon hubrichti*, a mountaintop isolate, was believed to be geographically restricted as a result of interspecific competition with wide-ranging *P. cinereus*. Using morphological, behavioral, resource use, climatic data, and ecological niche modeling, we found no evidence supporting the hypothesis that biotic interactions have restricted the range of *P. hubrichti*. There was no food resource partitioning or morphological divergence in sympatry, and *P. hubrichti* was more aggressive relative to *P. cinereus*. There was however, evidence that abiotic forces restrict the distribution of *P. hubrichti*. Local environmental variation was associated with population morphology, and there was sympatric morphological convergence. Both are consistent with local adaptation. Additionally, ecological niche modeling accurately predicted the range of *P. hubrichti*. Finally, niche modeling indicated that the ecologically-viable range of *P. cinereus* included 90% of the range of *P. hubrichti*, although it is absent from nearly this entire region.

---

[1] Assistant Professor, Department of Ecology, Evolution, and Organismal Biology, Iowa State University. Primary author and researcher.
[2] Graduate Student, Department of Ecology, Evolution, and Organismal Biology, Iowa State University. Primary author and researcher.
[3] Assisstant Professor, Department of Biology, University of Montevallo. Secondary author.

Combined with the behavioral data, these results suggest that interactions with *P. hubrichti* restrict the distribution of *P. cinereus,* the converse of what was predicted based upon species distributions alone. Our study emphasizes that the integration of multiple data types is an effective approach for understanding community organization.

Introduction

Understanding the forces that generate ecological communities and maintain species boundaries are major goals of community ecology. Extensive experimental and observational research has shown that biotic interactions such as competition[1-3] and predation[4,5] can be important forces regulating species coexistence, yet abiotic mechanisms such as disturbance[6,7], environmental and physical forces[8], and historical evolutionary events[9,10] also play key roles in governing community structure. However, while much is known about the effects of specific forces on particular communities, few studies have examined these forces simultaneously. As a result, little is known about how these forces interact to influence the evolution of community organization.

Because ecological communities are composites of the distributions of species that inhabit a particular region, their geographic ranges are also influenced by similar ecological and evolutionary forces. Gene flow from central to peripheral populations[11,12], the ability of peripheral populations to adapt to local conditions, interspecific competition[13], and abiotic conditions can all affect the range of a species. One way to examine the consequences of these forces is to estimate the fundamental niche from them and compare predicted ranges to actual species distributions[14]. Recently, bioclimatic modeling has been used to predict species' ranges and fundamental niches[15,16]. These methods use environmental and physical characteristics of known geographic locations to predict the ecologically-viable distribution of a species[17]. From these predictions, hypotheses regarding the mechanisms that maintain species' boundaries can then be tested[18]. Ecological niche modeling is a potent technique that complements standard examinations of biotic and abiotic influences on species' ranges and community structure. Therefore, combining these approaches should provide a powerful means of elucidating the forces influencing species' ranges, and for testing alternative hypotheses such as local adaptation or interspecific competition, and their effects on community organization.

The terrestrial salamanders of the genus *Plethodon* exhibit an intriguing geographic pattern that makes them ideal for employing this pluralistic approach to community organization. Throughout northeastern North America, the wide-ranging red-backed salamander (*P. cinereus*) completely surrounds the ranges of several sibling species. One such species is the Peaks of Otter salamander (*P. hubrichti*), which is geographically restricted to a range of less than 120 km^2 in the Blue Ridge Mountains of west-central Virginia[19] and has a narrow sympatric zone with *P. cinereus*. Both species appear to be territorial[20]. Based on this distributional pattern it has been hypothesized that *P. hubrichti* is geographically restricted as a result of interspecific competition with *P. cinereus*[21,22]. This hypothesis leads to two testable predictions. First, if biotic interactions are restricting the range of *P. hubrichti*, we predicted that *P. cinereus* is more interspecifically aggressive toward *P. hubrichti* than *P. hubrichti* is to *P. cinereus.* Additionally we predicted that the two species will partition food resources and diverge in cranial morphology when in sympatry. Both predictions are based on extensive experimental research in other communities of *Plethodon*, where interference (behavioral) competition[23-27] and exploitative competition[28,29] are dominant biotic forces regulating species' interactions. As an alternative, if abiotic forces are restricting the range of *P. hubrichti*, we predicted an association between local environmental characteristics (temperature and rainfall) and phenotypic traits across populations. Such a pattern provides evidence of adaptation to the local environment. We tested both the biotic and abiotic predictions simultaneously using a combination of morphological, behavioral, and ecological (food use) data. We then complemented these analyses with ecological niche modeling, to elucidate further the patterns and processes that operate to maintain these narrowly overlapping species' distributions.

Materials and Methods

We obtained a total of 571 adult specimens (251 *P. cinereus* and 320 *P. hubrichti*) from 18 localities across the range of *P. hubrichti* and the surrounding habitat occupied by *P. cinereus*. Specimens were obtained from the National Museum of Natural History (Washington D.C., USA), the Carnegie Museum of Natural History (Pittsburgh, Pennsylvania, USA), and from our own personal holdings collected in multiple years from the same set of sites (JAW: collected in 1993, DCA & SA: collected in 2004; see ref. 20 for

locality details). Two of these localities represented sympatric sites where both species were found, while the remaining 16 localities represented single species allopatric sites (8 localities per species).

From all specimens, head shape morphology was quantified using landmark-based geometric morphometrics methods[30,31]. These methods generate shape variables from the coordinates of biologically homologous points, after the effects of any differences in translation, rotation, and scale are mathematically held constant[32]. First we obtained digital images of the left-lateral side of each head using a Nikon DXM-1200 digital camera mounted on a Nikon SMZ1500 stereomicroscope, and digitized the location of eleven biologically-homologous landmarks from the skull and mandible of each (Fig. 1*a*). Variation in the position of the mandible relative to the skull was then standardized for all specimens[33], and a set of variables representing head shape was generated using standard morphometric techniques[34-36]. To quantify food resource use we removed the stomach contents from the specimens collected in 2004 (DCA & SA collection). Prey consumed by each salamander was recorded and classified to the level of Order[28,29]. To quantify behavior, we obtained data from the specimens collected in 1993 (JAW collection). Frequencies of aggressive and submissive behaviors were recorded from sixty 15-min. paired interspecific interactions in the laboratory [20]. Aggressive behaviors (look toward, move toward, all trunk raised, bite) and submissive behaviors (look away, move away, flat) were categorized based on previous studies of *Plethodon* behavior (see ref. 20 for citations). Finally, local environmental characteristics were quantified by extracting bioclimatic variables from the geo-referenced locations on a 1-km^2 resolution grid using DIVA-GIS[37]. The variables extracted for from DIVA-GIS included annual mean temperature, temperature seasonality (standard deviation x 100), annual mean precipitation, and precipitation seasonality (coefficient of variation), which were derived from the a compiled database of weather station data[37].

The hypothesis that biotic interactions are restricting the range of *P. hubrichti* was examined as follows. To test exploitative competition-based predictions, we assessed whether food resources were partitioned in sympatry using a G-test that compared resource use profiles of sympatric and allopatric populations of both species. We then assessed morphological differentiation between species (*P. cinereus* vs. *P. hubrichti*) and between

sites (allopatry vs. sympatry) using a two-way MANOVA. Differences in head shape among populations were visualized using a principal components analysis (PCA). Morphological divergence between species in sympatry relative to allopatry was then assessed using two permutation procedures[38]. The first examined observed sympatric differences to differences between randomly paired allopatric populations[28,38,39]. The second compared the observed divergence between allopatric and sympatric populations (D_{obs}) to the divergence between allopatric and sympatric populations where individuals were randomly assigned to populations (D_{rand} ; see ref. 38).

To test interference-based predictions, we compared the total frequency of aggressive behavior and the total frequency of submissive behavior exhibited by each species using Mann-Whitney U-tests. To test the hypothesis that abiotic forces are influencing the range of *P. hubrichti,* we used a multivariate association method (two-block partial-least squares)[40] to determine the degree of association between the four local environmental variables and the average morphology for each of the geographic localities. Finally, we used ecological niche modeling to examine whether the distributions of both species in this region are closely approximated by their predicted ecologically-viable distribution based on climatic variables[15,17]. Ecological-niche models were constructed using 19 bioclimatic indices that incorporated temperature and rainfall information across a 1-km^2 resolution grid over the study system (see ref. 37). We first predicted the ecologically-viable range of *P. hubrichti* (using 42 geo-referenced localities which mapped to 23 points on a 1-km^2 resolution grid) and statistically compared this to its current distribution[14]. We then predicted the ecologically-viable range of *P. cinereus* (using 1903 geo-referenced localities which mapped to 1744 points on a 1-km^2 resolution grid) and compared it to the current distribution of *P. hubrichti.* For both models, we used 19 bioclimatic indices across a 1-km resolution grid, and the BIOCLIM algorithm with a true/false criterion and a standard percentile cut-off in DIVA-GIS[37].

Results

We found no evidence to support the hypothesis that biotic interactions with *P. cinereus* have restricted the range of *P. hubrichti.* First, the two species did not partition food resources in sympatry as was expected from the exploitative competition hypothesis (G =

15.87, df = 15, P = NS). In both sympatry and allopatry, the dominant prey items for both species were Acarina, Annelida, Collembola, and Hymenoptera. Secondly, when morphology was examined we found significant phenotypic differentiation between species (Wilks' L = 0.54, F = 16.34, $P < 0.0001$) and between sites (Wilks' L = 0.157, F = 4.82, $P < 0.0001$), but sympatric phenotypic divergence was not greater than allopatric phenotypic divergence (P_{Rand} = NS) Instead, we found significant phenotypic convergence for both sympatric localities, where the observed sympatric phenotypic divergence was smaller than the divergence for 60 of 66 ($P < 0.0001$) and 43 of 66 ($P = 0.0047$) randomly paired allopatric populations (Fig. 1*b*). Finally, when interference competition was considered, we found that *P. hubrichti* was both significantly more aggressive (*P. hubrichti*: $\bar{X}$ = 23.6, *P. cinereus:* $\bar{X}$ = 11.7, N = 30, Z = 4.83, $P < 0.0001$) and significantly less submissive (*P. hubrichti*: $\bar{X}$ = 0.47, *P. cinereus:* $\bar{X}$ = 2.07, N = 30, Z = 3.88, $P < 0.0001$) than *P. cinereus* during interspecific encounters in the laboratory (Fig. 1*c*). In addition, *P. hubrichti* bit *P. cinereus* almost 7 times more frequently than *P. cinereus* bit *P. hubrichti* ($\bar{X}$ = 0.2 bites and $\bar{X}$ = 0.03 bites per 15-min. trial, respectively). These results were counter to what was predicted by the interference competition hypothesis[23], in which *P. cinereus* was predicted to be more aggressive and less submissive.

In contrast, we found evidence to support the hypothesis that abiotic interactions have affected the distribution of *P. hubrichti.* First, there was a significant association between the local environmental variables and average morphology for all 20 populations (r = 0.61, P = 0.05: Fig. 1*d*). Salamanders from regions with more rainfall had more robust lower jaws relative to the cranium, while salamanders from regions with higher temperature and more seasonal variation in temperature and rainfall had the opposite phenotype (Fig. 1*d*). This result implied that adaptation to the local environment was likely a contributing factor in phenotypic differentiation among populations. The significant phenotypic convergence of both species in sympatry lends further support to this hypothesis. Finally, using ecological niche modeling, we found that environmental climatic information was sufficient to predict the range of *P. hubrichti* (Fig. 2*a*). The ecologically-viable range of *P. hubrichti* provided a highly significant fit to the known distribution (X^2 = 12.6, $P < 0.0001$) and correctly

predicted 80% of the known localities for the species. In stark contrast, the predicted ecologically-viable range of *P. cinereus* encompassed 90% of the distribution of *P. hubrichti* (Fig. 2*b*), a region where *P. cinereus* is largely absent. Combined with the behavioral data, these results strongly suggest that biotic interactions with *P. hubrichti* negatively impact and restrict the distribution of *P. cinereus*. When these findings are considered simultaneously, their synthesis leads to the surprising inference that abiotic forces and local adaptation explain the restricted distribution of *P. hubrichti*, while biotic effects of *P. hubrichti* prevent *P. cinereus* from inhabiting regions for which it is otherwise ecologically suited.

Discussion

A long standing controversy in evolutionary ecology is whether abiotic or biotic forces are responsible for the regulation of ecological communities. While it is likely that a combination of these forces operate in most biological communities[41], few studies have examined their effects simultaneously. In this study we examined the role of biotic and abiotic forces in a terrestrial salamander community consisting of a wide-ranging species and a species with a limited range. We combined morphological, behavioral, resource use, and environmental data to test predictions generated from biotic and abiotic community-level hypotheses. We found no support for the hypothesis that biotic interactions restricted the range of *P. hubrichti*. Rather, its current geographic distribution is consistent with and predicted by abiotic forces. In contrast, we found that *P. cinereus* was competitively excluded from amenable habitat through biotic interactions with *P. hubrichti*. Thus both abiotic and biotic forces interact to regulate the distributions of salamanders in this ecological community.

Examining our findings in light of what is known about the ecological and evolutionary interactions of *P. cinereus* with geographically-restricted *Plethodon*[23,29] leads to several interesting observations. First, although these communities all display a similar geographic pattern of a wide-ranging species surrounding the distribution of a more geographically-restricted species, the specific forces that regulate these communities are not concordant. Prior experimental evidence has demonstrated that biotic forces (competition) are important in many *Plethodon* communities[23,27,29,42]. Our results demonstrate that abiotic forces must also be considered, as in some cases they play an important role in regulating salamander

community organization. Second, our study reveals the surprising result that *P. hubrichti* is not restricted by *P. cinereus,* but is instead significantly adapted to its local environment, and is well suited to its current range. A similar situation is found between *P. ouachitae*, an extremely aggressive mountaintop isolate, and the wide-ranging *P. albagula.* Here the smaller endemic species maintains its geographic range behaviorally, but does not expand its range at the expense of *P. albagula*[27]. Future research should determine whether *P. ouachitae* is similarly geographically restricted as a result of local adaptation to abiotic conditions.

The abiotic limit on the geographically-restricted and federally-designated species of concern *P. hubrichti* is in stark contrast to the federally endangered *P. shenandoah,* which is excluded from its preferable habitat as a result of competition with *P. cinereus*, and is thus restricted to sub-optimal talus habitat[23,42]. Because both species are of federal and local concern, comparisons between the two provide valuable insight into what management tactics may prove most effective. When viewed from this perspective, our results provide an alternative view of what is restricting *P. hubrichti* to its current range (environmental effects and not competition), which likely has profound implications for how to best maintain viable populations of this species. For instance, much of the range of *P. hubrichti* lies within an area that produces high-quality timber. In non-timbered sites, the diet quality of *P. hubrichti* is higher and tends to support larger population sizes[43]. Additionally, those sympatric sites that have been invaded by *P. cinereus* were either recently timbered, or are adjacent to recently timbered sites[19]. Because *P. hubrichti* is abiotically restricted to its present geographic range, timbering decisions by the National Forest Service have the potential to affect drastically the viability of this geographically restricted species.

Finally, our study demonstrates the necessity of employing a pluralistic approach to community ecology. Though much can be gained by examining the effects of biotic and abiotic forces individually, only by combining them can one determine how these forces interact to regulate communities. For the community that we examined, biotic forces appear to be dominant in regulating one species, while abiotic forces appear to be dominant in regulating the other species. This is a simple interaction between biotic and abiotic forces, yet without examining these forces concurrently it would have been difficult to ascertain how they regulate this community. It should be recognized, however, that more complicated

interactions between forces are likely operating in other ecological communities, and these interactions can only be elucidated by examining multiple causal forces simultaneously. It is now standard in evolutionary and ecological research to examine multiple response variables simultaneously using multivariate methods, so that the covariance between traits can be quantified and assessed. This approach has revealed that considerable biological information is found in the covariance between traits. In a similar manner, we believe that the covariance between causal variables should also be captured and examined, as it is likely that covariance between these forces is an important determinant of biological variability. The approach that we employed in this study is one means of capturing that covariance and gaining insight into how causal forces interact to regulate species distributions and community structure.

Acknowledgments

We thank Carl Anthony, Christopher Beachy, Michael Collyer, Robert Jaeger, Joseph Mitchell, and Nicole Valenzuela, who read and commented on earlier versions of this manuscript, and Pipat Rengusang and Robert Hijmans for assistance with GIS software. Carl Anthony, Robert Jaeger, and Joseph Mitchell provided assistance to JAW for the behavioral study. Specimens were collected under permits Virginia-SCP9348, US Forest Service (File code 2600), and US Dept. Interior (RM-29) (to JAW) and Virginia-023890, US Forest Service (File code 2600), and US Dept. Interior – BLRI-2004-SCI-0019, (to DCA). Finally, we are deeply indebted to Addison Wynn, whose patience and continual assistance in providing specimens from the collections of the Smithsonian made our research possible. This project was sponsored in part by National Science Foundation grants DEB-0122281 and DEB-0446758 (to DCA), DEB-9314081 (to R. G. Jaeger, National Geographic Society grant 5108-93 (to Robert G. Jaeger), and Society of Sigma Xi and the GSO at University of Louisiana at Lafayette (to JAW), and support from the Blue Ridge Parkway and the Jefferson National Forest (to JAW).

Literature Cited

1. Gurevitch, J., Morrow, L. L., Walsh, A., & Walsh, J. S. (1992) *Amer. Natur.* 140, 539—572.
2. Gurevitch, J., Morrison, J. A., & Hedges, L. V. (2000) *Amer. Natur.* 155, 435—453.
3. Schluter, D. (2000) *The ecology of adaptive radiation* (Oxford University Press, Oxford).

4. Sih, A., Englund, G. & Wooster, D. (1998) *Trends Ecol. Evol.* 13, 350—355.
5. Bertrand, A., Barbieri, M. A.., Cordova, J., Hernandez, C., Gomez, F., & Leiva, F. (2004) *ICES J. Mar. Sci.* 61, 1105-1112.
6. Levins, S. A., & Paine, R. T. (1974) *Proc. Natl. Acad. Sci. USA.* 71, 2744-2747.
7. Wootton, J. T. (2001) *Ecol. Lett.* 4, 46—56.
8. Pasachnik, S. & Ruthig, G. R. (2004) *J. Herpetol.* 38, 434-437.
9. Losos, J. B. (1992) *Syst. Biol.* 41, 403—420.
10. Losos J. B. (1996) *Ecology* 77, 1344—1354.
11. Hoffman, A. A., & Blows, W. (1994) *Trends Ecol. Evol.* 9, 223-227.
12. Kirkpatrick, M., & Barton, N. (1997) *Amer. Natur.* 150, 1—23.
13. Case, T. J., & Taper, M. L. (2000) *Amer. Natur.* 155, 583—605.
14. Peterson, A. T. (2001) *Condor.* 103, 599—605.
15. Nix, H. (1986) in *Atlas of elapid snakes of Australia.* ed. Longmore, R. (Australian Government Publishing Service, Canberra), pp. 4—15.
16. Stockwell, D. R. B., & Peters, D. P. (1999) *Intell. J. Geog. Inf. Syst.* 13, 143—158.
17. Raxworthy, C. J., Martinez-Meyer, E. Horning, N., Nussbaum, R. A., Schneider, G. E., Ortega-Huerta, M. A., & Peterson, A. T. (2003) *Nature.* 426, 837—841.
18. Cicero, C. (2004) *Evolution.* 58, 1573—1587.
19. Pague, C., & Mitchell, J. (1990) *Report to the Jefferson National Forest by the Virginia Department of Conservation and Recreation.* 16 pp.
20. Wicknick, J. A. (1995) Ph.D. dissertation (University of Louisiana, Lafayette, LA).
21. Highton, R. (1972) in *The Distributional History of the Biota of the Southern Appalachians. Part III: Vertebrates.* ed. Holt, P. C. (Virginia Polytechnic Institute, Blacksburg), pp. 139—188.
22. Jaeger, R. G. (1974) *BioScience.* 24, 33-39.
23. Jaeger, R. G. (1970). *Evolution* 24, 632—642.
24. Jaeger, R. G. (1971). *Ecology* 52, 632—637.
25. Nishikawa, K. C. (1985) *Evolution* 39, 1282—1294.
26. Nishikawa, K. C. (1987) *Anim. Behav.* 35, 263—270.
27. Anthony, C. D., Wicknick, J. A., & Jaeger, R. G. (1997) *Behav.* 134, 71—88.

28. Adams, D. C. (2000) in *The Biology of Plethodontid Salamanders,* eds. Bruce, R. C., Jaeger, R. G., & Houck, L. D. (Plenum, New York), pp. 383—393.
29. Adams, D. C., & Rohlf, F. J. (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97, 4106—4111.
30. Rohlf, F. J., & Marcus, L. F. (1993) *Trends Ecol.Evol.* 8, 129—132.
31. Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004) *Ital. J. Zool.* 71, 5—16.
32. Rohlf, F. J., & Slice, D. E. (1990) *Syst. Zool.* 39, 40—59.
33. Adams, D. C. (1999) *Evol. Ecol. Res.* 1, 959-970.
34. Bookstein, F. L. (1991) *Morphometric tools for landmark data: Geometry and biology.* (Cambridge Univ. Press, New York).
35. Dryden, I. L., & Mardia. K. V. (1998) *Statistical shape analysis.* (Wiley, New York).
36. Rohlf, F. J., & Bookstein, F. L. (2003) *Syst. Biol.* 52, 66—69.
37. Hijmans, R. J., Guarino, L., Bussink, C., Mathur, P., Cruz, M., Barrentes, I., & Rojas, E. (2004) *DIVA-GIS. Ver. 5.0. A geographic information system for the analysis of species distribution data.*
38. Adams, D. C. (2004) *Ecology.* 85, 2664-2670.
39. Schluter, D., & McPhail, J. D. (1992) *Amer. Natur.* 140, 85—108.
40. Rohlf, F. J., & Corti, M. (2000) *Syst. Biol.* 49, 740—753.
41. Wilbur, H. M. (1987) *Ecology* 68, 1437—1452.
42. Griffis, M. R., & Jaeger, R. G. (1998). *Ecology* 79, 2494-2502.
43. Mitchell, J. C., Wicknick, J. A., & Anthony, C. D. (1996) *Amphibian & Reptile Conservation* 1, 15-19.

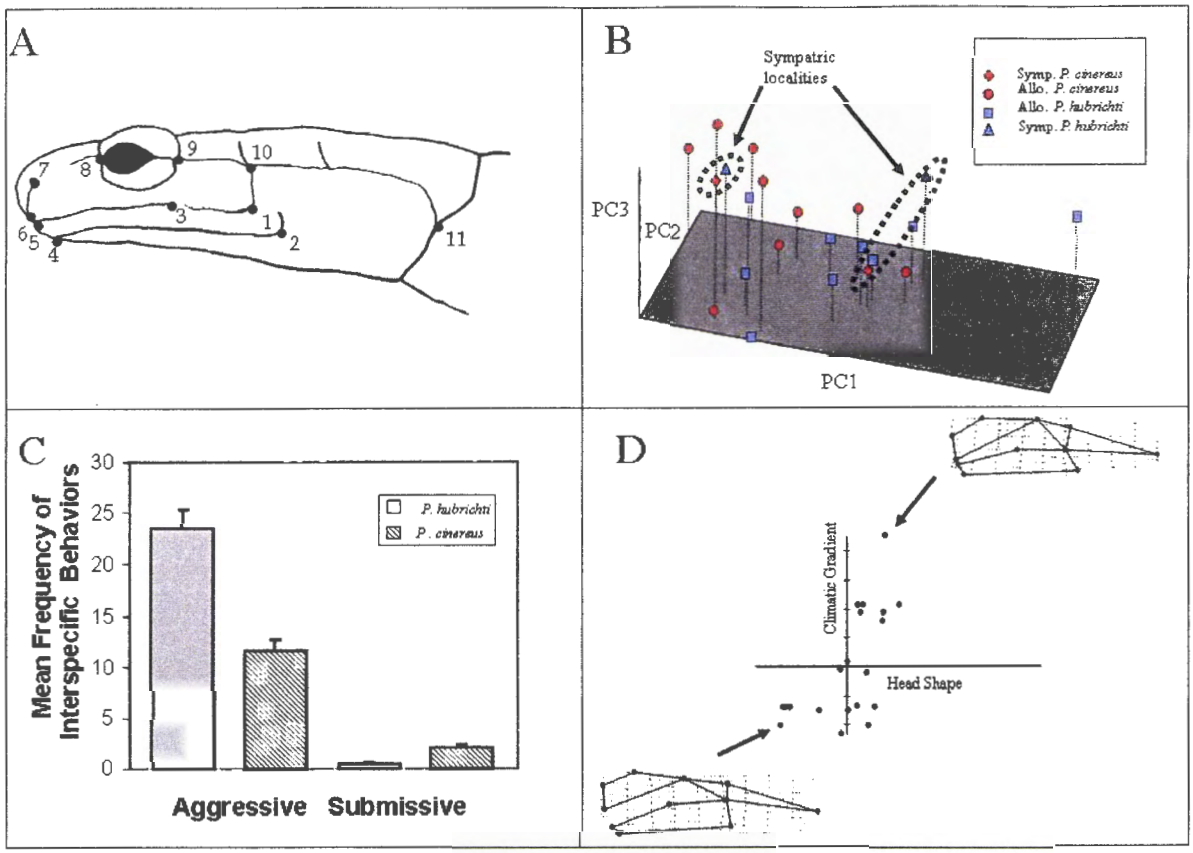

Fig. 1. (*A*) Positions of 11 landmarks used in this study. All landmarks were digitized from the left-lateral view of the skull (modified from Adams, ref. 38). (*B*) Principal components plot describing differences in head shape variation among allopatric and sympatric populations (3 PC axes explained 79% of the variation). Populations from sympatric sites indicated (population averages are shown). (*C*) Mean frequency of interspecific behaviors exhibited during laboratory aggression trials. (*D*) Multivariate association of head shape and environmental variation for the 20 populations used in this study. The *x* axis represents morphology (extremes illustrated by using a thin-plate spline), and the *y* axis represents environmental variation (positive values = high avg. temp and more variation in seasonality, negative values = higher average rainfall)

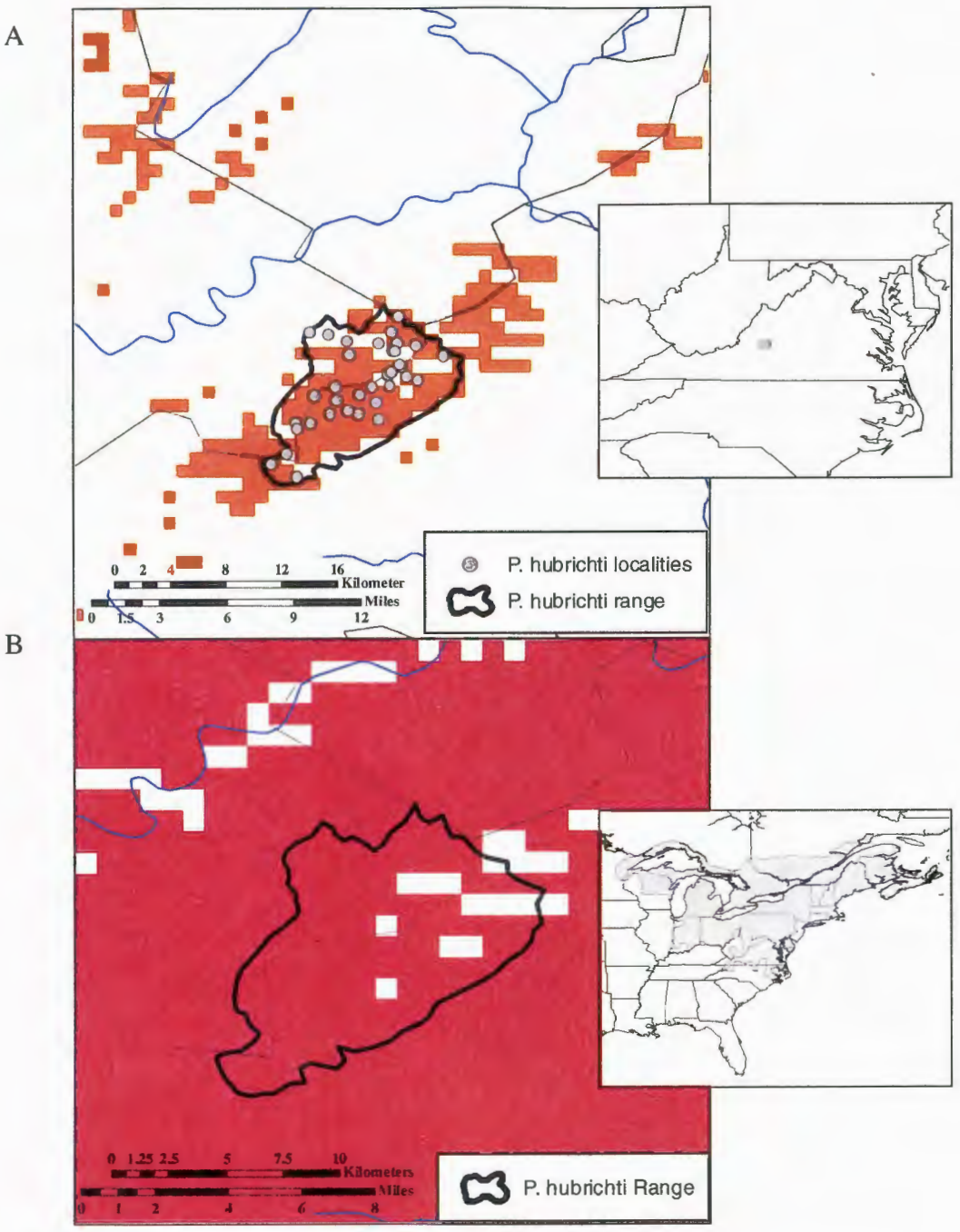

Fig. 2. (*A*) Ecologically-viable range for *P. hubrichti,* as predicted using the BIOCLIM algorithm (true/false criterion with 95 percentile cut-off). The current distribution of *P. hubrichti* is outlined in black. (*B*) Ecologically-viable range for *P. cinereus* (near the range of *P. hubrichti*), as predicted using the BIOCLIM algorithm (true/false criterion with 97.5 percentile cut-off). The current extent of *P. cinereus*'s range is shaded in grey (inset).

CHAPTER 3: GENERAL CONCLUSION

3.1 General Discussion

The role of biotic and abiotic factors in community development has been well investigated in evolutionary ecology. Research indicates that both factors play an integral part in evolution of community structure (Ricklefs and Miller 1999). It is probable that a combination of both factors determines community structure, however most research to this point has treated their effects as mutually exclusive hypothesis in regards to individual species distributions or multi-species communities (Case and Taper 2000, but see Wilbur 1987). Studying forces that regulate community structure from this either/or perspective may paint an inadequate picture of the system in question.

I examined a simple terrestrial salamander community consisting of a wide-ranging species and a species with a limited a range using a pluralistic approach. Hypotheses regarding both biotic (competition) and abiotic forces were tested using morphological, resource use (food) and environmental data. Previous knowledge on behavioral interaction and territoriality between the two species (Wicknick 1995) was also incorporated in understanding the community structure in this system. In addition, I also complemented above analysis with ecological-niche modeling to further ascertain the processes that maintain a stable community in the overlapping areas of the distributions of these species.

I found no evidence for the hypothesis that *Plethodon hubrichti*'s range is restricted as a result of competition with *Plethodon cinereus*, through either interference or exploitative competition. Additionally, ecological-niche models predicted a distribution for *P. hubrichti* that very closely approximated the observed distribution, suggesting that some environmental/abiotic factor is restricting *P. hubrichti*'s distribution. In contrast ecological-niche modeling for suitable habitat for *P. cinereus* predicted that all of *P. hubrichti*'s current range is viable for *P. cinereus*. Prior behavioral studies between the two species demonstrate as *P. hubrichti* being the more aggressive and least submissive of the two species (Wicknick

1995).It is thus clear that *P. cinereus* is excluded from *P. hubrichti*'s current range via aggressive behavioral interactions. Furthermore, I found a significant correlation between average morphology and four environmental variables for all sampled populations in the area, which further corroborated the hypothesis of an abiotic limitation for *P. hubrichti.* Thus, this simple community provides a concrete example of regulation of community structure via interactions of both abiotic and biotic factors.

This study emphasizes the need for pluralistic approaches to inferring factors that determine community structure. Using an either/or approach to testing community-level hypothesis may not be beneficial in gaining an understanding of the larger picture for any given community. In this instance we find that one species in the community is regulated largely by biotic forces (the exclusion of *P. cinereus* from *P. hubrichti's* range) whereas abiotic forces dominate regulation of the other species (local adaptation of *P. hubrichti*). Only by approaching the question by integrating multiple data sets can we begin to decipher how both forces interact to regulate community structure.

3.2 Implications for *Plethodon* Community Ecology

As mentioned in chapter 1, the study of *P. hubrichti* and *P. cinereus* community ecology represents only one chapter in elucidating the evolutionary community ecology of the genus *Plethodon*. However, the pattern of communities resulting from distributional overlap of wide-ranging and limited ranges species is apparent all across the distribution of the genus. This pattern has been instrumental in hypothesizing that these communities are largely regulated through competition (Highton 1972). Evidence for competition in maintaining community structure in *Plethodon* has been well demonstrated but this competition may be induced either through exploitative (Adams and Rohlf 2000) or interference means (Jaeger 1974). Hence, studies of *Plethodon* community ecology have largely been based around the biotic hypothesis. An essential component added by this study, to the larger study of *Plethodon* community ecology, is to incorporate hypothesis concerning abiotic forces. The

role of abiotic forces, along with biotic ones, in community ecology is well demonstrated in this single system and is likely prevalent in others as well.

3.3 Conservation and Management of *Plethodon hubrichti*

Owing to it's severely restricted range *P. hubrichti* was considered to be a rare species (Pague and Mitchel 1987). In 1989 it was designated as a species of special concern. This designation resulted from the both the small range and the fact that not much about the species was known. Several studies in the 1990's have furthered our understanding of the biology of *P. hubrichti* (Pague and Mitchell 1990, Wicknick 1995, Mitchell et al. 1996).

An important consideration for designating the species as one of special concern is apparent in comparison with the species *P. shenandoah*. The latter is a sibling species with a similar endemic range restricted to three mountain peaks in the Shenandoah National Park. As with *P. hubrichti*, the entire range of *P. shenandoah* is surrounded by *P. cinereus*, and the latter is known to exclude *P. shenandoah* from more preferable habitat (Jaeger 1974, Griffis and Jaeger 1998). Hence, *P. shenandoah* has been considered in danger of extinction due to competitive exclusion from *P. cinereus* and been designated as a federally endangered species. However, unlike *P. shenandoah*, *P. hubrichti* does not appear to be restricted due to competitive interactions with *P. cinereus*. The limited range of *P. hubrichti* is likely resultant from local adaptation to the environment. Moreover, no evidence of *P. cinereus* encroaching upon *P. hubrichti's* range has been demonstrated (Aasen and Reichenbach 2004) and densities of *P. hubrichti* within its range appear to be quite healthy (personal observation).

There does appear to be some cause of concern with the destruction and human disturbance of the species' native habitat. A large portion of *P. hubrichti*'s distribution coincides with areas of high timber quality sites owned by the National Forest Service. Mitchell et al. (1996) demonstrated that *P. hubrichti* from non-timbered sites had a better diet quality. Additionally, sites that were recently clear-cut supported relatively smaller population sizes as compared to non-timbered sites or shelterwood cutting sites. Reducing canopy cover through logging or defoliation through gypsy moths leads to drying of leaf

litter and humus layer. This drying can be detrimental to salamander movements and foraging ability (Jaeger 1990, Jager and Bernard 1981). Further more, all five sympatric localities where *P. cincerus* co-occurs within the range of *P. hubrichti* are either at or adjacent to sites that have been either recently logged or adjacent (Pague and Mitchell 1990). This suggests that timbering may provide a backdoor access to *P. cinereus* in effectively invading *P. hubrichti*'s range. Therefore, timbering and logging activities in the area must be tightly regulated and monitored in order to not jeopardize the future of this endemic species.

3.4 Considerations for Future Research

Even though it appears that *P. hubrichti*'s distribution is related to abiotic factors and there is evidence for local adaptation, the exact mechanism is yet known. Of particular importance is to determine the exact physiological mechanism that restricts the range of the species. Whether this restriction is associate with moisture, temperature or some other abiotic factor. In particular moisture has been demonstrated as an essential factor in determining distributions of closely related taxa (Hairston 1949, Grover 2000). Relationships of the distribution with temperature and soil pH may also be insightful.

In determining the evolution of *P. hubrichti*'s range it would be wise to conduct hypothesis regarding local adaptation. Mayr (1963) posited that populations on the periphery may be restricted from expanding by not adapting to their local conditions to the disruptive influence of gene flow from the center. Several studies have shown that gene flow in a patchy environment may keep populations maladapted to their local conditions. (see Camin and Ehlrich 1958, Endler 1977,, Reichert 1993, Dias 1996). The theoretical framework for evaluating such hypotheses has been well developed in recent years (Kirkpatrick and Barton 1997, Case and Taper 2000). A simple and initial hypothesis to test local adaptation would be to measure rates of gene flow from central to peripheral populations. High rates of gene flow would indicate that populations at the periphery are restricted from adapting to their local conditions and hence the species is held back from expanding its current range. Studying

rates of gene flow and how they interact with local selection pressure will be extremely helpful in determining why the range of *P. hubrichti* is so extremely restricted.

3.5 Literature Cited

Aasen, G., and Reichenbach, N. 2004. Is the red-backed salamander (*Plethodon cinereus*) encroaching upon populations of the Peaks of Otter Salamander (*P. hubrichti*)? *Catesbeiana*. 24:17-20.

Adams, D. C., and F. J. Rohlf. 2000. Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences, U.S.A.* 97:4106-4111.

Camin, J.H. and P. Ehlrich. 1958. Natural selection in water snakes (*Natrix sipedon* L.) on islands in Lake Erie. *Evolution* 12:504-511

Case, T. J., and M. L. Taper. 2000. Interspecific competition, environmental gradients, gene flow, and the coevolution of species borders. *American Naturalist.* 155:583–605.

Dias, P.C. 1996. Sources and sinks in population biology. *Trends in Ecology and Evolution.* 11:326-330.

Endler, J.A. 1977. *Geographic variation, speciation, and clines*. Princeton University Press, Princeton, N.J.

Griffis, M. R., and Jaeger, R. G. 1998. Competition Leads to an Extinction-Prone Species of Salamander: Interspecific Territoriality in a Metapopulation. *Ecology.* 79: 2494-2502.

Grover, M.C. 2000. Determinants of salamander distribution along moisture gradients. *Copeia.* 2000:156-168.

Hairston, N.G. 1949. The local distributions and ecology of the Plethodontid salamanders of the southern appalachians. *Ecological Monographs.* 19:47-73.

Highton, R. 1972. Distributional interactions among eastern North American salamanders of the genus *Plethodon*. In *The distributional history of the biota of the southern Appalachians. Part III: vertebrates.* (Holt, P.C., ed.). Pp 139-188. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Jaeger, R.G. 1990. Territorial salamanders evaluate size and chitinous content of arthropod prey. In *Behavioral Mechanisms of Food Selection* (Hughes, R.N. ed.). Springer-Verlag, Heidelberg.

Jaeger, R.G. 1974. Competitive exclusion: comments on survival and extinction of species. *Bioscience.* 24:33-39.

Jaeger, R.G. and D.E. Bernard. 1981. Foraging tactics of a terrestrial salamander: choice of diet in structurally simple environments. *American Naturalist.* 117:639-664.

Kirkpatrick, M. and N.H. Barton. 1997. Evolution of a species range. *American Naturalist.* 150:1-23.

Mayr, E. 1963. *Animal species and evolution*. Harvard University Press, Belknap, Cambridge, Mass.

Mitchell, J.C., J.A. Wicknick, and C.D. Anthony. 1996. Effects of timber harvesting practices on Peaks of Otter salamanders (*Plethodon hubrichti*) populations. *Amphibian and Reptile Conservation* 1:15-19.

Pague, C.A. and J.C. Mitchell. 199.0. The distribution of the Peaks of Otter salamander (*Plethodon hubrichti*). Unpublished report submitted to Jefferson National Forest by the Virginia Department of Conservation and Recreation, Division of Natural Heritage.

Ricklefs, R.E., and G.L. Miller. 1999. *Ecolo*gy. 4th ed. W.H. Freeman and Company, New York, New York, U.S.A.

Riechert, S.E. 1993. The evolution of behavioral phenotypes: lessons learned from divergent spider populations. In *Advances in the study of behavior* (Slater, P.J.B., J.S. Rosenblat, C.T. Snowdon, and M. Milinski eds.). Pp 103-134. Vol. 22. Academic Press, San Diego, CA.

Wicknick, J. A. 1995. Interspecific competition and territoriality between a widespread species of salamander and a species with a limited range. Ph.D. Dissertation. University of Southwestern Louisiana, Lafayette, LA.

Wilbur, H.M. 1987. Regulation of Structure in Complex Systems: Experimental Temporary Pond Communities. *Ecology.* 68: 1437:1452.